The International Agency for Research on Cancer (IARC) Monographs Programme identifies environmental causes of cancer in humans and has evaluated more than 900 agents in the last few decades. The Monographs Programme evaluates chemicals, complex mixtures, occupational exposures, physical agents and biological agents, as well as personal habits. Monographs are written by a Working Group (WG) over a period of about 12 months to evaluate all of the scientific literature on a given substance and, through a transparent and rigorous process[1], reach a decision on the degree to which the scientific literature supports the ability of that substance to cause cancer. For Monograph 112[2], 17 expert scientists evaluated the carcinogenic hazard for 4 insecticides and the herbicide glyphosate. The WG concluded that glyphosate was a probable human carcinogen. This finding stirred great debate globally on the safety of glyphosate and led to a careful evaluation of the IARC monograph results when they came available on July 29, 2015. On August 31, 2015, the German Federal Institute for Risk Assessment (BfR) completed an addendum[3] (the BfR Addendum) to the Draft Renewal Assessment Report[4] (RAR) for glyphosate. This addendum was leaked by the media[5]. The Addendum draws a very different conclusion on the literature than did the IARC WG. We are seriously concerned about the scientific quality of the BfR Addendum and feel that it is misleading regarding the potential for a carcinogenic hazard from exposure to glyphosate. We are also concerned about some of the implications of the Addendum regarding the use of human data in identifying carcinogenic hazards.

Our comments to the BfR Addendum will focus on the human evidence, the animal laboratory evidence and the mechanistic evidence.

The Human Evidence

The BfR agrees with the IARC WG that there is "limited evidence in humans for the carcinogenicity of glyphosate". In the IARC review process, this is defined as "A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence."[1] The BfR Addendum (p. ii) then characterizes the IARC interpretation as "precautionary" and that the BfR takes a more "cautious view" of this classification because "no consistent positive association was observed", "the most powerful study showed no effect" and that the studies "could not differentiate between the effects of glyphosate and the co-formulants". We will consider the first two arguments here and target the third argument for the end of our letter.

The finding of "limited evidence" by the IARC WG was for non-Hodgkins lymphoma (NHL). High-quality cohort studies are particularly valuable for determining the carcinogenicity of an agent because their design can facilitate exposure assessment and reduce the potential for certain biases. The Agricultural Health Study (AHS) was the only cohort study available providing information on the carcinogenicity of glyphosate. The study had a very weak positive finding for NHL (RR 1.1, 0.7-1.9) with no apparent

exposure response in the results. The BfR refers to this study as "the most powerful study" and that it was negative for NHL.

Several theoretical limitations of case-control studies are laid out in epidemiology textbooks [6, 7]. The BfR uses these limitations to label all of the case-control studies as unreliable. This gives the impression that all of the studies are equal in quality and unusable for an overall evaluation. This is not the case: well-designed case-control studies are recognized as an efficient alternative to cohort studies [7]. An IARC WG carefully evaluates all of the available epidemiology data, looking at the study's strengths and weaknesses as well as the study order. This is key in determining whether the positive associations seen are a reliable indication of an association or simply due to chance or methodological flaws. To provide a reasonable interpretation of the findings, an evaluation needs to properly weight studies according to their quality rather than simply count the number of positives and negatives. The meta-analysis cited in the IARC Monograph[8] and redone by the WG is an excellent example of an objective evaluation of the existence of a consistent positive trend; this meta-analysis showed a statistically significant association. The BfR provided no justification for their evaluation of "no consistent positive association".

The final BfR conclusion (p. 22) that "there was no unequivocal evidence for a clear and strong association of NHL with glyphosate" is misleading. IARC, like many other groups, uses three levels of evidence for human data[1]. "Sufficient Evidence" means "that a causal relationship has been established" between glyphosate and NHL. The BfR conclusion can be rewritten to mean that the epidemiological data does not meet the criteria for "Sufficient Evidence" established by IARC. However, this says nothing about concern that would arise for an association that is not strong enough to be causal, but is strong enough that "that causality is credible" as does the IARC "Limited Evidence" category.

Evidence from Chronic Exposure Animal Studies

We are astonished by the conclusions of the BfR regarding the animal carcinogenicity data. The IARC WG review found a significant positive trend for renal tumors in CD-1 mice[9], a rare tumour. A significant positive trend means that as the exposure increases, the pattern seen in the data supports an increasing risk with increasing dose. No comparisons of any individual exposure group to the control group were significant. The WG also identified a significant positive trend for hemangiosarcoma in male CD-1 mice[10], again with no individual exposure group significantly different from controls. Finally, the WG also saw a significant increase in the incidence of islet cell adenomas in two studies in Sprague-Dawley rats[11-13]. In one of these rat studies, thyroid adenomas in females and liver adenomas in males were also increased. Thus, glyphosate was positive for malignant tumors in both of the mice studies examined and for benign tumors in 2 of the five rat studies examined. By the IARC review criteria[1], the evidence in the mouse constitutes sufficient evidence in animals.

The BfR agreed, stating (p. 44) "it is obvious that IARC concludes on "sufficient evidence of carcinogenicity" because the criteria for this conclusion are fully met." The IARC WG

reached this conclusion using data that were publicly available in sufficient detail for independent scientific evaluation (a requirement of the IARC Preamble[1]). Based on the BfR Addendum, it seems there were 3 additional mouse studies and 2 additional rat studies where they had sufficient evidence to review the findings. BfR reported on two additional studies with a positive trend for renal tumors, one in CD-1 mice[14], and one in Swiss-Webster mice[15]. One of these studies[14] also reported a positive trend for hemangiosarcoma. Moreover, BfR reported two studies in CD-1 mice showing significant trends for malignant lymphoma[14, 16]. For all of the tumors described above in CD-1 mice, a positive trend was seen against the concurrent control.

However, in all cases in CD-1 mice, including those observed by the IARC, the BfR dismisses the observed trends in tumour incidence because there are no individual treatment groups which are significantly different from controls and because the maximum observed response is within the range of the historical control data (Table 5.3-1 in the Addendum). Care must be taken in using historical control data to evaluate animal carcinogenicity data. In virtually all guidelines[1, 17], scientific reports[18] and publications[19-21] on the issue, the first choice should be the use of the concurrent controls. For instance, the Preamble to the IARC Monographs states, "it is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls…". When using historical control data, it should be from the same timeframe for the exact strain, preferably from the same laboratory or the same supplier and preferably with the same pathologist[17]. This was not the case for the historical control database used by BfR. One of the mouse studies[9] was clearly done before this historical control database was developed, one study[14] used Crj:CD-1 mice rather than Crl:CD-1 mice, and 1 study[10] did not specify the substrain and was reported in 1993 (probably started prior to 1988); hence only a single study [16] used the right strain, but was reported more than 10 years after the historical control dataset was developed. Interestingly, the historical control data used by the BfR [22] was from studies in 7 laboratories using the Charles River Laboratory CD1 mice. Surprisingly, there is a second report [23] by the same authors with a larger control database using the same mouse strain from 11 laboratories over the same time period (1987-2000) showing very different results. For example, the 2000 publication[22] shows 5 and 4 studies out of 46 with adenomas (no more than 2 in any one study) and adenocarcinomas (one in each study) respectively whereas the 2005 report[23] shows only 1 study each out of 54 studies with a single adenoma and a single adenocarcinoma; all other studies had no tumors.

Given this evidence, it is hard to perceive how the BfR reached the conclusion they provided. By their own evaluation, there were seven (7) positive findings in mice with three replicates for one tumor type and 2 positive findings for carcinomas in rats. After discarding the inappropriate use of historical evidence, it is no longer scientifically justifiable to refer to all of these studies as negative.

Mechanistic Information

The BfR Addendum dismisses the WG finding that "there is strong evidence that

glyphosate causes genotoxicity" by suggesting that the evidence not seen by the IARC WG was overwhelmingly negative and that, since the studies that were reviewed were not done under guideline principles, they should get less weight. To maintain transparency, IARC reviews use only publicly available data. Thus it is impossible for any scientists not associated with BfR to review this conclusion with any degree of scientific certainty. On the other hand, the BfR did not include evidence from exposed humans that was highlighted in the IARC Monograph.

The BfR confirms (p. 79) that the studies evaluated by the IARC WG on oxidative stress were predominantly positive but do not agree that this is strong support for an oxidative stress mechanism. They reduce the significance of these findings predominantly because of a lack of positive controls in some studies and because many of the studies used glyphosate formulations and not pure glyphosate. The WG concluded that (p. 77) "Strong evidence exists that glyphosate, AMPA and glyphosate-based formulations can induce oxidative stress". From a scientific perspective, these types of mechanistic studies can play a key role in distinguishing between the effects of mixtures, pure substances and metabolites and we would encourage the BfR to carefully review this science.

Finally, we strongly disagree that literature data should automatically receive less weight than guideline studies; once a chemical is on the market, the majority of the research done on that chemical will be done by very competent research laboratories that will use unique models to address specific issues related to toxicity that will not have guidelines associated with them. These have great value in understanding mechanisms of carcinogenicity and should be given appropriate weight in an evaluation based on study quality and not just guideline rules.

General Comments

Science moves forward based on data, careful evaluation of that data and a rigorous review of the findings. One important aspect of this process is transparency and on the ability to question the findings of others. This insures the credibility of the results and provides a strong basis for decisions. Many of the aspects of transparency do not exists for the BfR RAR [4] or the Addendum[3]. There are no authors or contributors listed for either document, a requirement for virtually all scientific papers. Citations for almost all of the references, even those from the open scientific literature, have been redacted from the documents. The ability to objectively evaluate the findings of a scientific report requires a complete list of the supporting evidence.

A second important aspect of the scientific process is a careful evaluation and analysis of the facts. Guidelines have been devised for analyzing carcinogenicity data developed after careful consideration of scientists on a global basis. One of the most widely cited is [17] which is cited in the BfR Addendum. This document gives guidance on the analysis of carcinogenicity studies in contradiction to the methods used by the BfR. Thus, BfR uses the concept of guidelines to rule out the substantive

inclusion of literature data into their risk assessment, but ignores guidelines when it comes to the use of historical controls and trend analyses.

Summary

The IARC WG concluded that glyphosate is a "probable human carcinogen" putting it into IARC category 2A. In their 2013 Draft RAR, BfR concluded (Vol. 1, p. 139) "classification and labeling for carcinogenesis is not warranted" and "glyphosate is devoid of genotoxic potential". How is this possible? Consider the evidence and the conclusions.

The IARC WG saw an association between NHL and glyphosate in the human evidence, but could not rule out chance, bias and confounding; the IARC definition of "limited evidence"[1] for epidemiological data. BfR agreed, noting that other IARC categories are "not suitable". However the BfR concluded that an association was seen but dismissed it as insufficiently consistent.

The IARC WG saw significant effects for two tumors in two mouse studies and benign tumors in two rat studies. The BfR confirmed the statistically significant findings by the IARC WG, and agreed that the IARC criteria of "sufficient" evidence in animals is "fully met". BfR went on to identify two more mouse studies with kidney tumors, a second mouse study with an increase in hemangiosarcoma, and two mouse studies showing increases in malignant lymphoma. Thus, all five mouse studies examined by the BfR were positive in at least 1 tumor site, 1 was positive in 3 tumor sites. Then using an inappropriate historical control dataset in an inappropriate way, dismiss all of these findings as chance.

The IARC WG concluded strong evidence of genotoxicity and oxidative stress for glyphosate, entirely from publicly available data, including data on DNA damage in blood of exposed humans. The BfR, while confirming the positive studies seen for genotoxicity dismissed them all because they were not guideline studies and because, in their interpretation, all of the guideline assays were negative. The BfR confirmed the positive studies seen for oxidative stress, noted some concern over these data, but concluded they could not use them because there were no other data to support a finding of carcinogenicity or genotoxicity and the mechanism cannot stand alone.

We feel that the scientific arguments supporting the BfR review of the human, animal and mechanistic evidence is fundamentally flawed and should be rejected. We are concerned that this evaluation appears to have been designed to achieve a pre-determined goal rather than an objective scientific review. Finally, we strongly object to the almost non-existent weight given to studies from the literature by the BfR and the strong reliance on non-publicly available data in a limited set of assays that define the minimum data necessary for the approval of a pesticide.

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